Applicants: KHVOROVA et al. Serial No.: 10/714,333

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AMENDMENTS

Amendments to the Claims:

This listing replaces all prior versions and listings of claims in the application:

Listing of Claims:

- (previously introduced) A method for selecting a siRNA for a target gene, wherein said siRNA comprises 19 – 25 nucleotide base pairs, said method comprising the steps:
 - (a) selecting a target gene;
 - (b) generating a set of candidate siRNA molecules, wherein each of said candidate siRNA molecules comprises an antisense region of at least 19 bases that is at least 79% complementary to a region of the target gene;
 - (c) applying a criterion to each of said candidate siRNA molecules, wherein the criterion is selected from the group consisting of: (i) the number of A and U nucleotides present in the first five nucleotide positions at the 5' terminus of the antisense region is higher than that present in the last five nucleotide positions at the 3' terminus of the antisense region; (ii) the number of A and U nucleotides present in the first four positions at the 5' terminus of the antisense region is higher than that present in the last four positions at the 3' terminus of the antisense region; (iii) the number of A and U nucleotides present in the first three positions at the 5' terminus of the antisense region is higher than that present in the last three positions at the 3' terminus of the antisense region; (iv) the number of A and U nucleotides present in the first two positions at the 5' terminus of the antisense region is higher than that present in the last two positions at the 3' terminus of the antisense region; and (v) the first 5' position of the antisense region has either an A or U nucleotide and the last 3' position of the antisense region has neither an A nor U nucleotide; and

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- (d) selecting a candidate siRNA from the set of candidate siRNA molecules of step (b) as said siRNA for the target gene, if said candidate siRNA satisfies said criterion.
- 2. (canceled)
- 3. (canceled)
- 4. (canceled)
- 5. (canceled)
- 6. (canceled)
- 7. (canceled)
- 8. (canceled)
- 9. (canceled)
- 10. (canceled)
- 11. (canceled)
- 12. (canceled)
- 13. (canceled)
- 14. (canceled)
- 15. (canceled)
- 16. (canceled)
- 17. (canceled) 18. (canceled)
- 19. (canceled)
- 20. (canceled)
- 21. (canceled)
- 22. (canceled)
- 23. (canceled)
- 24. (canceled) 25. (canceled)
- 26. (canceled) 27. (canceled)
- 28. (canceled)

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29. (canceled)

30. (canceled)

(canceled)

32. (canceled)

33. (canceled)

34. (canceled)

35. (canceled)

36. (canceled)

37. (canceled)

- 38. (previously presented) The method according to claim 1, wherein the criterion is: the number of A and U nucleotides present in the first five nucleotide positions at the 5' terminus of the antisense region is higher than that present in the last five nucleotide positions at the 3' terminus of the antisense region.
- 39. (previously presented) The method according to claim 1, wherein the criterion is: the number of A and U nucleotides present in the first four positions at the 5' terminus of the antisense region is higher than that present in the last four positions at the 3' terminus of the antisense region.
- 40. (previously presented) The method according to claim 1, wherein the criterion is: the number of A and U nucleotides present in the first three positions at the 5' terminus of the antisense region is higher than that present in the last three positions at the 3' terminus of the antisense region.
- 41. (previously presented) The method according to claim 1, wherein the criterion is: the number of A and U nucleotides present in the first two positions at the 5' terminus of the antisense region is higher than that present in the last two positions at the 3' terminus of the antisense region.

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42. (previously presented) The method according to claim 1, wherein the criterion is: the first 5' position of the antisense region has either A or U and the last 3' position of the antisense region had neither A nor U.

- 43. (previously presented) A method for selecting a siRNA for a target gene, wherein said siRNA comprises 19 – 30 nucleotide base pairs, said method comprising the steps:
 - (a) selecting a target gene;
 - (b) generating a set of candidate siRNA molecules, wherein each of said candidate siRNA molecules comprises a sense region of 19 – 30 bases, wherein said sense region comprises a sense sequence of 19 bases that is at least 79% similar to a region of the target gene;
 - (c) applying a set of one or more criteria selected from the group consisting of the presence of A at position 19 of the sense sequence, the presence of A at position 3 of the sense sequence, the presence of U at position 10 of the sense sequence, the presence of A at position 14 of the sense sequence, the absence of C at position 19 of the sense sequence, the absence of G at position 13 of the sense sequence, the absence of U at position 5 of the sense sequence and the absence of A at position 11 of the sense sequence, wherein said sense sequence occupies positions 1 19 of the sense region, and wherein when said siRNA is 20 30 base pairs, bases that are not within said sense sequence occupy positions 11 to 11 and positions 1 to 11 are located at the 5' end of the sense region; and
 - (d) selecting a candidate siRNA from the set of candidate siRNA molecules of step (b) as said siRNA for the target gene, if said candidate siRNA satisfies said one or more criteria.
- 44. (previously presented) The method according to claim 43, wherein the set of one or more criteria includes the presence of A at position 19 of the sense sequence.

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- 45. (previously presented) The method according to claim 43, wherein the set of one or more criteria includes the presence of A at position 3 of the sense sequence.
- 46. (previously presented) The method according to claim 43, wherein the set of one or more criteria includes the presence of U at position 10 of the sense sequence.
- 47. (previously presented) The method according to claim 43, wherein the set of one or more criteria includes the presence of A at position 14 of the sense sequence.
- 48. (previously presented) The method according to claim 43, wherein the set of one or more criteria includes the absence of C at position 19 of the sense sequence.
- 49. (previously presented) The method according to claim 43, wherein the set of one or more criteria includes the absence of G at position 13 of the sense sequence.
- 50. (previously presented) The method according to claim 43, wherein the set of one or more criteria includes the absence of U at position 5 of the sense sequence.
- 51. (previously presented) The method according to claim 43, wherein the set of one or more criteria includes the absence of A at position 11 of the sense sequence.
- 52. (previously presented) The method according to claim 43 further comprising applying one or more additional criteria selected from the group consisting of: a GC content between about 30% and 52%, at least 2 A or U bases at positions 15 19 of the sense sequence, and a internal repeat that is not stable at greater than 50°C, and selecting said candidate siRNA if said candidate siRNA satisfies said one or more additional criteria.
- 53. (previously presented) The method according to claim 43, wherein said candidate siRNA is selected as said siRNA for the target gene if said candidate siRNA satisfies at least two criteria selected from the group consisting of: the presence of A at

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position 19 of the sense sequence, the presence of A at position 3 of the sense sequence, the presence of U at position 10 of the sense sequence, the presence of A at position 14 of the sense sequence, the absence of C at position 19 of the sense sequence, the absence of G at position 13 of the sense sequence, the absence of U at position 5 of the sense sequence, and the absence of A at position 11 of the sense sequence.

- 54. (previously presented) The method according to claim 43, wherein said candidate siRNA is selected as said siRNA for the target gene if said candidate siRNA satisfies at least three criteria selected from the group consisting of: the presence of A at position 19 of the sense sequence, the presence of A at position 3 of the sense sequence, the presence of U at position 10 of the sense sequence, the presence of A at position 14 of the sense sequence, the absence of C at position 19 of the sense sequence, the absence of U at position 5 of the sense sequence, and the absence of A at position 11 of the sense sequence.
- 55. (previously presented) The method according to claim 43, wherein said candidate siRNA is selected as said siRNA for the target gene if said candidate siRNA satisfies at least four criteria selected from the group consisting of: the presence of A at position 19 of the sense sequence, the presence of A at position 3 of the sense sequence, the presence of U at position 10 of the sense sequence, the presence of A at position 19 of the sense sequence, the absence of C at position 19 of the sense sequence, the absence of G at position 19 of the sense sequence, the absence of U at position 5 of the sense sequence, the absence of A at position 11 of the sense sequence, a GC content between about 30% and 52%, at least 2 A or U bases at positions 15 19 of the sense sequence, and a internal repeat that is not stable at greater than 50°C.
- 56. (previously presented) The method according to claim 43, wherein said candidate siRNA is selected as said siRNA for the target gene if said candidate siRNA satisfies

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at least five criteria selected from the group consisting of: the presence of A at position 19 of the sense sequence, the presence of A at position 3 of the sense sequence, the presence of U at position 10 of the sense sequence, the presence of A at position 14 of the sense sequence, the absence of C at position 19 of the sense sequence, the absence of G at position 19 of the sense sequence, the absence of G at position 13 of the sense sequence, the absence of U at position 5 of the sense sequence, the absence of A at position 11 of the sense sequence, a GC content between about 30% and 52%, at least 2 A or U bases at positions 15 – 19 of the sense sequence, and a internal repeat that is not stable at greater than 50°C.

- 57. (previously presented) The method according to claim 43, wherein said candidate siRNA is selected as said siRNA for the target gene if said candidate siRNA satisfies each of the following criteria: a GC content between about 30% and 52%, at least 2 A or U bases at position 15-19 of the sense sequence, A at position 19 of the sense sequence, A at position 3 of the sense sequence, a base other than C at position 19 of the sense sequence, a base other than G at position 13 of the sense sequence, and an internal repeat that is not stable at a temperature of greater than 50°C.
- 58. (previously presented) The method according to claim 43, wherein said candidate siRNA is selected as said siRNA for the target gene if said candidate siRNA satisfies each of the following criteria: a GC content of between 30% and 52% and a base other than G at position 13 of the sense sequence.
- 59. (previously presented) The method according to claim 43, wherein said candidate siRNA is selected as said siRNA for the target gene if said candidate siRNA satisfies each of the following criteria: a base other than C at position 19 of the sense sequence and a base other than G at position 13 of the sense sequence.
- 60. (previously presented) The method according to claim 43, wherein said candidate siRNA is selected as said siRNA for the target gene if said candidate siRNA satisfies each of the following criteria: a GC content of between 30% and 52%, a base other

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than G at position 13 of the sense sequence and an internal repeat that is not stable at a temperature of greater than 50°C.